

RESEARCH ARTICLE

Lung function assessment in the Pacific walrus (*Odobenus rosmarus divergens*) while resting on land and submerged in water

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ABSTRACT

In the present study, we examined lung function in healthy resting adult (born in 2003) Pacific walruses (*Odobenus rosmarus divergens*) by measuring respiratory flow (\dot{V}) using a custom-made pneumotachometer. Three female walruses (670–1025 kg) voluntarily participated in spirometry trials while spontaneously breathing on land (sitting and lying down in sternal recumbency) and floating in water. While sitting, two walruses performed active respiratory efforts, and one animal participated in lung compliance measurements. For spontaneous breaths, \dot{V} was lower when walruses were lying down (e.g. expiration: $7.1 \pm 1.2 \text{ l s}^{-1}$) as compared with in water ($9.9 \pm 1.4 \text{ l s}^{-1}$), while tidal volume (V_T , $11.5 \pm 4.6 \text{ l}$), breath duration ($4.6 \pm 1.4 \text{ s}$) and respiratory frequency ($7.6 \pm 2.2 \text{ breaths min}^{-1}$) remained the same. The measured V_T and specific dynamic lung compliance ($0.32 \pm 0.07 \text{ cmH}_2\text{O}^{-1}$) for spontaneous breaths were higher than those estimated for similarly sized terrestrial mammals. V_T increased with body mass (allometric mass-exponent=1.29) and ranged from 3% to 43% of the estimated total lung capacity (TLC_{est}) for spontaneous breaths. When normalized for TLC_{est} , the maximal expiratory \dot{V} (\dot{V}_{exp}) was higher than that estimated in phocids, but lower than that reported in cetaceans and the California sea lion. \dot{V}_{exp} was maintained over all lung volumes during spontaneous and active respiratory manoeuvres. We conclude that location (water or land) affects lung function in the walrus and should be considered when studying respiratory physiology in semi-aquatic marine mammals.

KEY WORDS: Pulmonary function testing, Spirometry, Tidal volume, Respiratory flow, Lung compliance, Marine mammals

INTRODUCTION

Marine mammals have to balance available O_2 while using aerobic metabolism during foraging dives and are required to return to the surface to replenish their O_2 stores and remove CO_2 . This necessitates efficient gas exchange to rapidly replenish the consumed O_2 before commencing the next dive. The functional and mechanical adaptations of the respiratory system are crucial for gas exchange, and the scientific effort to describe and better understand these features in marine mammals has progressively increased since the first works of Irving (1939) and Scholander

(1940). Past work has highlighted the anatomical and functional differences when comparing the respiratory system of marine mammals with that of their terrestrial counterparts (for reviews, see Fahlman et al., 2017; Kooyman, 1973; Piscitelli et al., 2013). For example, studies on the respiratory function in both pinnipeds and cetaceans showed that the tidal volume (V_T , l) and respiratory frequency (f_R , breaths min^{-1}) are, respectively, higher and lower in marine as compared with terrestrial mammals (Fahlman et al., 2017). Also, these studies revealed that, unlike terrestrial mammals, marine mammals are able to generate high respiratory flow (\dot{V} , l s^{-1}) during breaths of short duration (Fahlman et al., 2017). In addition, the structure and function of the respiratory system in marine mammals is important in the determination of their diving limitations (Bostrom et al., 2008; Fahlman et al., 2009), and increasing environmental impacts of the ocean (e.g. decreased prey availability or underwater sonar) could lead to an alteration in their diving behaviour. Therefore, an increased understanding of respiratory function in different species is important to gain a better knowledge of the physiological limitations on these species.

The respiratory physiology of the walrus (*Odobenus rosmarus*) has been poorly investigated and data only exist on f_R in wild Atlantic walruses (*Odobenus rosmarus rosmarus*: Bertelsen et al., 2006; Stirling and Sjare, 1988), or f_R and end-expired alveolar CO_2 on one animal under professional care (Mortola and Limoges, 2006; Mortola and Seguin, 2009). Spirometry is a minimally invasive method that provides knowledge on basic respiratory function and mechanics (Burki, 1981; Crapo, 1994), and has recently been adapted for use in voluntarily participating pinnipeds and cetaceans (Fahlman et al., 2020a,b; Fahlman et al., 2019a,b, 2015; Fahlman and Madigan, 2016; Kooyman and Cornell, 1981; Matthews, 1977; Reed et al., 2000). In the present study, we aimed to increase basic respiratory physiology data in the walrus by measuring lung function and mechanics in voluntarily participating adult females using spirometry. A previous study that investigated the California sea lion (*Zalophus californianus*) suggested that the location (resting in water or on land) could affect respiratory function as a result of pressure on the chest (Fahlman et al., 2020b). Thus, we tested the hypothesis that body position on land (sitting or lying down in sternal recumbency) or floating in water would significantly alter lung function variables [e.g. V_T , f_R , total breath duration (T_{tot} , s) and \dot{V}]. We also hypothesized that expiratory \dot{V} (\dot{V}_{exp}) is maintained over most of the vital capacity (VC , l) in the walrus, and that lung compliance (C_L , $\text{l cmH}_2\text{O}^{-1}$) is higher as compared with that of terrestrial mammals, as previously reported for other marine mammal species (Fahlman et al., 2017).

Our results show that in the walrus, \dot{V} increases when floating in water, while V_T , T_{tot} and f_R remain the same in both media. \dot{V} , V_T , T_{tot} and dynamic C_L were similar to those reported for other pinnipeds, where V_T increased with body mass (M_b , kg) with an allometric mass-exponent close to 1. The flow–volume relationship showed a nearly constant \dot{V}_{exp} over all lung volumes while

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List of symbols and abbreviations

C_L	lung compliance
f_R	respiratory frequency
M_b	body mass
MAV	minimum air volume
P_{amb}	ambient atmospheric pressure
P_{ao}	airway opening pressure
P_{oeso}	oesophageal pressure
P_L	transpulmonary pressure
sC_L	specific lung compliance
$s\dot{V}$	specific respiratory flow
$s\dot{V}_E$	mass-specific respiratory minute volume
$s\dot{V}_{exp}$	specific expiratory flow
$s\dot{V}_{insp}$	specific inspiratory flow
sV_T	mass-specific tidal volume
T_{exp}	expiratory duration
T_{insp}	inspiratory duration
T_{tot}	total breath duration
TLC	total lung capacity
TLC_{est}	estimated total lung capacity
VC	vital capacity
VC_B	behavioural vital capacity
\dot{V}	respiratory flow
\dot{V}_E	respiratory minute volume
\dot{V}_{exp}	expiratory flow
\dot{V}_{insp}	inspiratory flow
V_T	tidal volume
$V_{T,exp}$	expiratory tidal volume
$V_{T,insp}$	inspiratory tidal volume

performing spontaneous and active breaths. In addition, when normalized for the estimated total lung capacity (TLC_{est} , l), \dot{V}_{exp} from active respiratory manoeuvres was higher than that seen in other pinnipeds, but lower than that reported in cetaceans and in the California sea lion.

MATERIALS AND METHODS

Study subjects

Three adult Pacific female walruses [*Odobenus rosmarus divergens* (Illiger 1815)], born in 2003 and housed under professional care at the Oceanogràfic (Valencia, Spain), participated in the present study (Table 1). The animals were rescued in the wild as orphan pups and were brought into the aquarium. The health of the walruses was assessed daily, and no pulmonary disease was detected during the data collection period.

Procedures, morphometrics and environmental parameters

The walruses were desensitized to the equipment and trained to perform the experimental procedures using operant conditioning. Therefore, participation in each research trial was voluntary, where

the animals could end the experimental trial at any time. This procedure allowed for data collection in a relaxed physiological state. Spirometry trials while spontaneously breathing were performed while inactive in three different body positions: (1) lying down in sternal recumbency, (2) sitting while supported by the pectoral flippers and (3) floating on the water surface (see fig. 1 in Fahlman et al., 2020b). The walruses adopted a vertical position while floating in a 3 m deep seawater pool. The estimated water height acting on the centroid of the lungs was approximately 40 cm. Two of the walruses were trained to perform 5–10 consecutive maximal respiratory efforts while sitting on land. The animals performed these manoeuvres at their individual capacity and we will refer to them as active respiratory efforts/manoeuvres, where the maximum measured V_T was considered as the behavioural vital capacity (VC_B , l). In addition, one walrus was trained to swallow an oesophageal balloon catheter that allowed measurement of the dynamic C_L during spontaneous breaths while sitting.

Data were collected from a total of 120 trials performed from February 2015 to July 2018 (2015: $n=25$, 2016: $n=1$, 2017: $n=52$, 2018: $n=42$), and a subset of 5 trials for each animal and body position (15 trials per position, $n=45$) were selected for the lung function analysis. Only trials where the walruses were resting for at least 2 min while performing complete breaths (composed of an exhalation and an inhalation) were included in the analysis. In addition, a total of 9 separate trials while sitting included active respiratory manoeuvres, and 3 separate trials measured dynamic C_L . The M_b of each animal was recorded in the same week as lung function testing, and the mean (\pm s.d.) for selected trials was 822 ± 96 kg (range 670–1025 kg). For the trials included in the study, the mean (\pm s.d.) ambient pressure was 101.4 ± 0.5 kPa (range 99.2–102.4 kPa), while the air temperature and humidity (thermometer and hygrometer OH513 Oh Haus & Co.) were $21.3 \pm 3.1^\circ\text{C}$ (16–27.6°C) and $74.1 \pm 8.3\%$ (57–99%), respectively. The water temperature at the facility housing the animals was $15.9 \pm 0.9^\circ\text{C}$ (14.5–20.1°C). All experiments were approved by the Animal Care and Welfare Committee of Fundació Oceanogràfic de la Comunitat Valenciana (animal care number: OCE-19-16) and the US Navy Bureau of Medicine and Surgery (BUMED NRD-910).

Respiratory flow measurements

\dot{V} was measured using a custom-made Fleisch type pneumotachometer (Mellow Design, Valencia, Spain) with a dead space of 700 ml. A soft silicone ring at the base of the pneumotachometer was designed to fit comfortably around the nostrils of the walrus. During data collection, the pneumotachometer was placed over the snout and gently pressed down to prevent leaks around the silicone base. The walrus was trained to close its mouth during data collection to ensure respiration only through the nostrils, and the trainer positioned one hand over the mouth, which allowed any possible leaks to be detected. A low-resistance laminar flow matrix (Z9A887-2, Merriam Process Technologies,

Table 1. Trial and sample details, morphometrics and respiratory frequency of Pacific walruses

Animal ID	No. of trials	No. of spontaneous breaths	No. of active breaths	Trial duration (min)	$M_{b,LF}$ (kg)	$M_{b,Exp}$ (kg)	TLC_{est} (l)	f_R (breaths min ⁻¹)
26005388	38	237	16	3.4±0.8	715±17	674±45	54.0±3.3	7.9±1.9 (5.5–12.2)
26005389	39	288	10	4.0±1.0	931±43	917±76	71.7±5.4	8.4±2.0 (4.2–10.7)
26005390	43	238	—	4.3±1.4	821±38	807±44	63.7±3.2	6.6±2.5 (3.1–10.8)

Animal ID of three voluntarily participating adult (birth year 2003) female Pacific walruses (*Odobenus rosmarus divergens*), total number of trials (including training sessions and analysed trials), total number of analysed spontaneous and active breaths, mean (\pm s.d., $n=45$) total trial duration, body mass ($M_{b,LF}$) measured the same week as lung function testing (± 0 –4 days), body mass measured during the experimental period ($M_{b,Exp}$), estimated total lung capacity (TLC_{est}) based on a previous allometric equation for marine mammals ($TLC_{est}=0.135M_b^{0.92}$, where M_b is in kg and TLC_{est} in l; Kooyman, 1973), and average and range for measured respiratory frequency (f_R) during the experimental procedure.

Cleveland, OH, USA) was placed inside the pneumotachometer, which created a resistance that increased with \dot{V} . This resistance resulted in a pressure difference across the flow matrix that was measured with a differential pressure transducer (Spirometer Pod, ML 311, ADInstruments, Colorado Springs, CO, USA) connected to the pneumotachometer via two firm-walled, flexible tubes of 310 cm length and 2 mm internal diameter (i.d.) (see details in Fahlman et al., 2015). The pneumotachometer was calibrated for linearity between flow and resistance before and after each trial by pumping different air flow rates, using a 7 l calibration syringe (Series 4900, Hans-Rudolph Inc., Shawnee, KS, USA) as previously detailed (Fahlman et al., 2019b).

Airway and oesophageal pressure measurements

For estimating the dynamic C_L , we measured the oesophageal (P_{oeso}) and airway opening pressures (P_{ao} , cmH₂O) during spontaneous breaths (Fahlman et al., 2015; Olsen et al., 1969). An oesophageal balloon catheter (47-9005, Cooper Surgical, Trumbull, CT, USA) was manually inserted into the oesophagus for measuring the P_{oeso} , and a sample port was placed above the nostrils of the walrus for measuring the P_{ao} . The catheter was placed at the level of the heart and inflated with 1.0 ml of air. Both sample lines were connected to a differential pressure transducer (MPX-100 mbar type 339/2, Harvard Apparatus, Holliston, MA, USA) through 288 cm length and 2 mm i.d., firm-walled and flexible tubes.

Data acquisition and processing

Measured differential pressures were passed through an amplifier (TAM-A Transducer Amplifier Module, Harvard Apparatus). The data were captured at 400 Hz using a data acquisition system (Powerlab 8/35, ADInstruments) and displayed on a laptop computer running LabChart (v. 8.1, ADInstruments).

Measured inspiratory \dot{V} (\dot{V}_{insp}) and \dot{V}_{exp} were integrated to estimate inspiratory ($V_{\text{T,insp}}$) and expiratory ($V_{\text{T,exp}}$) tidal volume as previously detailed (Fahlman et al., 2019b; Fahlman et al., 2015). All volumes were converted into standard temperature and pressure dry (STPD; Quanjer et al., 1993), where inhaled air was corrected for ambient humidity and temperature, and exhaled air was assumed to be at 37°C and 100% saturated with water vapour. f_R was calculated for each trial using the number of complete breaths divided by the measurement period. The dynamic C_L was estimated as $V_{\text{T,insp}}$ divided by the tidal change in transpulmonary pressure (P_L , cmH₂O; $P_L = P_{\text{ao}} - P_{\text{oeso}}$) measured at zero flow, following previous procedures (see fig. 1B in Fahlman and Madigan, 2016). The reference pressure for both P_{ao} and P_{oeso} was the ambient atmospheric pressure (P_{amb}).

Statistical analysis

For the statistical analysis of spontaneous breaths, only periods of normal and complete breaths were considered, and single exhalations or inhalations were removed as in previous studies

(Fahlman and Madigan, 2016; Fahlman et al., 2020b). For analysis of active breaths, we only included the 2–4 largest and most similar manoeuvres for each trial.

The difference between expiratory and inspiratory \dot{V} , V_T and duration for spontaneous (paired t -test) and active breaths (Wilcoxon signed-rank test) were analysed using SPSS (v.24.0, released 2016, IBM SPSS Statistics for Windows, IBM Corp., Armonk, NY, USA). The relationship between measured dependent variables (\dot{V} , V_T and f_R) while spontaneously breathing and M_b was analysed using linear mixed-effects models using the lme function in R (v.3.6.1, <http://www.R-project.org/>). The individual animal was treated as a random effect, which accounted for the correlation between repeated measurements on the same individual (Littell et al., 1998). Homoscedasticity for all models was confirmed by the Bartlett test, and in case of unequal variances the variable was log₁₀-transformed. Best models of remaining variables were chosen by the log-likelihood (LL) ratio test. The effect of the experimental factor (body position) on measured dependent variables (\dot{V} , V_T , T_{tot} and f_R) for spontaneous breaths was analysed using a two-way mixed-effects ANOVA (SPSS), where body position was treated as a fixed effect and individual animal as a random effect (Frederick, 1999). In this study $P \leq 0.05$ was considered as significant, and data are presented as means \pm s.d.

RESULTS

For lung function measurements from the three walruses while they were resting and spontaneously breathing in all body positions, the average trial duration was 3.9 ± 1.1 min (range 2.0–7.2 min, $n=45$) with a total of 763 complete breaths (Table 1). For separate trials while walruses were sitting, a total of 26 active respiratory manoeuvres from 9 trials and two animals were included in the analysis (Table 1), and 21 spontaneous breaths from 3 trials were used to estimate the dynamic C_L in one individual. The breathing pattern in the walrus began with an expiration followed by an inspiration and an end-inspiratory pause.

Inspiratory and expiratory phases

For each animal, the mean T_{tot} , expiratory (T_{exp}) and inspiratory (T_{insp}) duration, \dot{V}_{exp} , \dot{V}_{insp} , $V_{\text{T,exp}}$ and $V_{\text{T,insp}}$ for spontaneous breaths are reported in Table 2, and for active respiratory manoeuvres the same variables are reported in Table 3.

For spontaneous breaths, the average T_{tot} was 4.6 ± 1.4 s, where T_{exp} was significantly shorter (2.1 ± 0.7 s) than T_{insp} (2.5 ± 0.9 s, paired t -test, $t=-16.2$, d.f.=762, $P<0.01$). Mean \dot{V}_{exp} was significantly higher (8.5 ± 2.8 l s⁻¹) than \dot{V}_{insp} (6.4 ± 1.9 l s⁻¹, $t=25.3$, d.f.=762, $P<0.01$). V_T did not differ between expiration (11.5 ± 4.6 l) and inspiration (11.7 ± 4.6 l, $t=-1.74$, d.f.=762, $P>0.05$), and we therefore only report $V_{\text{T,exp}}$ when referring to V_T for spontaneous breaths, unless otherwise specified.

For active respiratory manoeuvres, average T_{tot} was 3.6 ± 0.9 s, and T_{exp} was significantly shorter (0.9 ± 0.3 s) than T_{insp} (2.7 ± 0.8 s, Wilcoxon signed-rank test, $T=351$, $P<0.01$), while average

Table 2. Lung function for spontaneous breaths in adult Pacific walruses

Animal ID	\dot{V} (l s ⁻¹)		V_T (l)		T (s)		
	Expiratory	Inspiratory	Expiratory	Inspiratory	Expiratory	Inspiratory	Total
26005388	9.1 \pm 2.5 (2.9–16.7)	5.6 \pm 1.5 (2.4–9.2)	9.6 \pm 3.0 (2.6–17.0)	9.7 \pm 2.8 (3.4–17.7)	1.7 \pm 0.6	2.5 \pm 0.8	4.2 \pm 1.3
26005389	9.0 \pm 2.6 (3.1–19.5)	7.0 \pm 1.8 (1.9–11.8)	12.7 \pm 5.0 (3.4–28.6)	12.8 \pm 5.1 (3.5–28.8)	2.0 \pm 0.6	2.4 \pm 0.9	4.4 \pm 1.2
26005390	7.1 \pm 2.8 (1.7–16.0)	6.5 \pm 2.0 (2.8–14.7)	12.0 \pm 4.9 (1.8–27.1)	12.2 \pm 4.9 (2.2–26.9)	2.5 \pm 0.7	2.7 \pm 0.9	5.2 \pm 1.4

Animal ID, mean (\pm s.d., $n=763$) and range for all body positions (sitting supported by the front flippers, lying down, and floating at rest in water) of expiratory and inspiratory flow (\dot{V}), tidal volume (V_T), and average duration (T) for each respiratory phase and total breath duration obtained from three voluntarily participating female walruses (*O. rosmarus divergens*) while spontaneously breathing.

Table 3. Lung function for active breaths in adult Pacific walruses

Animal ID	\dot{V} (l s ⁻¹)		V_T (l)		T (s)		
	Expiratory	Inspiratory	Expiratory	Inspiratory	Expiratory	Inspiratory	Total
26005388	29.2±5.3 (21.9–38.3)	10.2±1.4 (6.7–11.4)	19.4±4.0 (9.8–25.0)	19.6±3.4 (13.5–24.6)	1.0±0.2	2.9±0.8	3.9±0.9
26005390	46.6±5.2 (39.5–55.4)	11.3±2.0 (9.4–16.0)	17.8±8.0 (10.1–28.9)	20.2±7.8 (10.7–31.9)	0.6±0.2	2.5±0.7	3.1±0.8

Animal ID, mean (±s.d. $n=26$) and range of expiratory and inspiratory flow (\dot{V}), tidal volume (V_T), and average duration (T) for each respiratory phase and total breath duration of active respiratory manoeuvres obtained from two voluntarily participating female Pacific walruses (*O. rosmarus divergens*) while sitting and supported by their pectoral flippers.

\dot{V}_{exp} was significantly higher (35.9 ± 10.0 l s⁻¹) than \dot{V}_{insp} (10.3 ± 0.8 l s⁻¹, $T=0$, $P<0.01$). There were no differences between $V_{T,\text{exp}}$ (18.8 ± 5.7 l) and $V_{T,\text{insp}}$ (19.8 ± 5.4 l, $T=194$, $P>0.5$).

Study of body position

For the 45 selected trials to assess lung function from spontaneous breaths, T_{tot} did not significantly change with the body position of the animals (sitting 5.4 ± 1.3 s, lying 4.9 ± 0.8 s, water 3.9 ± 0.8 s, two-way mixed-effects ANOVA, $F_{2,4}=3.92$, $P>0.1$; Fig. 1). The body position of the animals significantly affected \dot{V} (expiration: $F_{2,4}=18.60$, $P<0.01$; inspiration: $F_{2,4}=10.42$, $P<0.05$), where both \dot{V}_{exp} and \dot{V}_{insp} were significantly lower when lying down (expiration: 7.1 ± 1.2 l s⁻¹; inspiration: 5.5 ± 1.1 l s⁻¹) as compared with when in water (expiration: 9.9 ± 1.4 l s⁻¹; inspiration: 7.2 ± 1.2 l s⁻¹, Tukey *post hoc* test, $P<0.001$ for all; Fig. 1). Mass-specific V_T (s V_T , ml kg⁻¹) did not significantly differ between the three body positions (sitting 15.7 ± 2.6 ml kg⁻¹, lying down 13.3 ± 2.5 ml kg⁻¹, water 13.8 ± 2.8 ml kg⁻¹, $F_{2,4}=2.7$, $P>0.1$). Similarly, no differences were detected between body positions for f_R (sitting 6.2 ± 2.3 breaths min⁻¹, lying down 8.3 ± 1.7 breaths min⁻¹, water 8.4 ± 2.0 breaths min⁻¹, $F_{2,4}=6.07$, $P>0.05$).

General lung function and dynamic lung compliance

For all body positions and spontaneous breaths, the average f_R for all trials was 7.6 ± 2.2 breaths min⁻¹ (Table 1). The average s V_T

while spontaneously breathing was 13.9 ± 5.2 ml kg⁻¹ (range 2.2 – 32.7 ml kg⁻¹), and the highest \dot{V} (19.5 l s⁻¹) and the largest V_T (28.8 l, s $V_T=32.7$ ml kg⁻¹) were measured while floating at the water surface (animal ID: 26005389; Table 2). TLC_{est} was calculated based on data from excised lungs of different species of marine mammals, including juvenile walruses ($TLC_{\text{est}}=0.135M_b^{0.92}$, where M_b is in kg and TLC_{est} is in l; Kooyman, 1973), using the M_b measured during the experimental period (Table 1). The average TLC_{est} was 63.2 ± 8.3 l, and for the three body positions and spontaneous breaths, the V_T ranged from 3% to 43% of the TLC_{est} . When performing active respiratory manoeuvres while sitting, the maximal \dot{V} (55.4 l s⁻¹) and the largest V_T (31.9 l) were measured for walrus 26005390 (Table 3), and the V_T reached 50% of TLC_{est} .

For spontaneous breaths, there was a positive correlation between $V_{T,\text{exp}}$ and $V_{T,\text{insp}}$ and M_b , with a mass-exponent close to unity (Fig. 2, Table 4). During spontaneous breathing, neither \dot{V}_{exp} nor

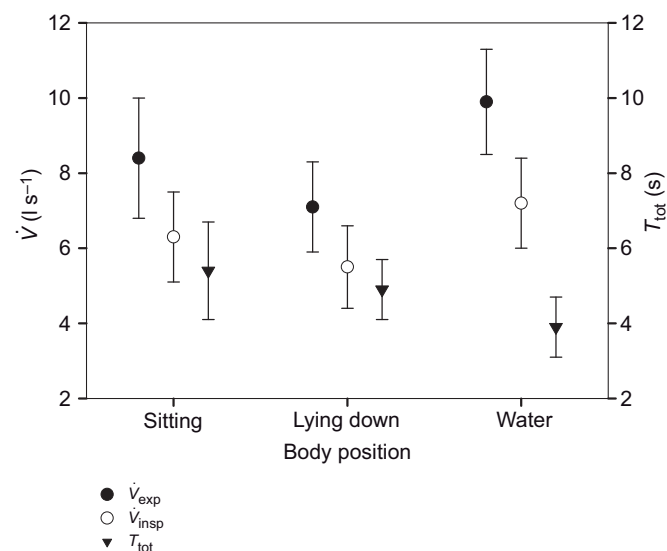


Fig. 1. Respiratory flow (\dot{V}) and total breath duration of spontaneous breaths from adult Pacific walruses in different body positions. Mean (±s.d., $n=15$ for each position) expiratory (\dot{V}_{exp}) and inspiratory flow (\dot{V}_{insp}), and total breath duration (T_{tot}) measured from three voluntarily participating female Pacific walruses (*Odobenus rosmarus divergens*) while sitting and supported by the front flippers (sitting), lying down in sternal recumbency (lying down) and in water (water).

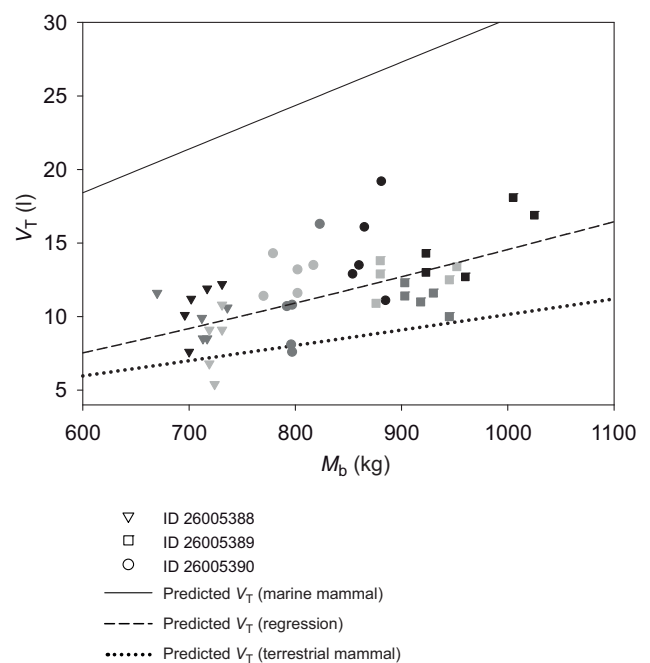


Fig. 2. Relationship between body mass and measured tidal volume during spontaneous breathing in adult Pacific walruses. Measured body mass (M_b) and tidal volume (V_T) from three voluntarily participating female Pacific walruses (*O. rosmarus divergens*) in three different body positions: sitting supported by the pectoral flippers (black), lying down (dark grey) and floating in water (light grey). The dashed line represents the regression line based on reported results in Table 4 for expiratory tidal volume ($V_{T,\text{exp}}$). The solid line shows the predicted V_T using the allometric equation obtained from a number of marine mammal species ($V_T=0.0372M_b^{0.97}$; Fahlgren et al., 2020b), while the dotted line represents the estimated V_T for terrestrial mammals at rest ($V_T=7.69M_b^{1.04}$; Stahl, 1967).

Table 4. Statistical results for linear mixed-effects models for voluntary breaths in adult Pacific walruses

Dependent	β_0	$\log_{10}(M_b)$	χ^2	P-value
$\log_{10}(V_{T,exp})$	-2.72 ± 0.80	1.29 ± 0.27	10.8	<0.01
$\log_{10}(V_{T,insp})$	-2.54 ± 0.82	1.24 ± 0.28	10.4	<0.01
\dot{V}_{exp}	—	—	<0.01	0.99
\dot{V}_{insp}	—	—	2.5	>0.10
$\log_{10}(f_R)$	9.20 ± 2.78	-2.86 ± 0.96	4.2	<0.05

Linear mixed-effects models including expiratory (exp) and inspiratory (insp) tidal volume (V_T , l), respiratory flow (\dot{V} , l s⁻¹) and respiratory frequency (f_R , breaths min⁻¹) measured for all body positions (sitting supported by the front flippers, lying down, and floating at rest in water) and spontaneous breaths. V_T , f_R and body mass (M_b , kg) were transformed using the base 10 logarithm (\log_{10}).

\dot{V}_{insp} correlated with M_b (Table 4). There was a positive correlation between f_R and M_b (Table 4).

The average dynamic C_L measured in one walrus (animal ID: 26005388) while spontaneously breathing was 1.09 ± 0.23 l cmH₂O⁻¹. As C_L varies with lung size (Stahl, 1967), the specific C_L (sC_L , cmH₂O⁻¹) was computed by dividing C_L by the minimum air volume (MAV, l), which was estimated to be 7% of total lung capacity (TLC, l), based on previous experiments with excised lungs (Fahlman et al., 2011). The M_b of walrus 26005388 at the time of the measurements was 640 kg, resulting in an average dynamic sC_L of 0.32 ± 0.07 cmH₂O⁻¹.

The range of \dot{V}_{exp} and \dot{V}_{insp} for spontaneous and active breaths combined was 1.7 – 55.4 l s⁻¹ and 1.9 – 16.0 l s⁻¹, respectively (Tables 2 and Table 3). When \dot{V}_{exp} and \dot{V}_{insp} were normalized to TLC_{est} (specific respiratory flow: $s\dot{V}$, s⁻¹), the $s\dot{V}$ for expiration ($s\dot{V}_{exp}$) ranged from 0.03 to 0.87 s⁻¹ and that for inspiration ($s\dot{V}_{insp}$) ranged from 0.03 to 0.25 s⁻¹. The flow–volume relationship for two animals while sitting showed that \dot{V}_{exp} and \dot{V}_{insp} were constant over all lung volumes during both spontaneous and active breaths (Fig. 3).

DISCUSSION

The respiratory variables collected in the present study were within the previous ranges measured for other marine mammal species and differed from that reported for terrestrial mammals (see a comparative summary in Table 5). The walruses showed a lower f_R than that expected for a similarly sized terrestrial mammal. The breathing pattern in the walruses was similar to that reported in other pinnipeds (Kooyman, 1973), and began with an expiration, followed by an inspiration and a respiratory pause. Our results showed that \dot{V} was lower while lying down than when floating in water, while sV_T , T_{tot} and f_R remained the same for all body positions. Measured V_T was higher than that estimated for a terrestrial mammal of similar size, increased with M_b with a mass-exponent close to unity, and reached 50% of TCL_{est} during active respiratory manoeuvres. Measured dynamic sC_L in one walrus was higher than that reported for terrestrial mammals, and was similar to that previously measured in pinnipeds and cetaceans (Fahlman et al., 2017). The peak $s\dot{V}_{exp}$ for active breaths was lower than that reported for cetaceans and California sea lions, but higher than that estimated for Weddell and grey seals. The flow–volume relationship showed that both \dot{V}_{exp} and \dot{V}_{insp} are maintained over the entire lung volume when performing both spontaneous and active respiratory manoeuvres.

Studies on respiratory function and mechanics in marine mammals have used different approaches. Some studies have used anaesthetized or post-mortem animals and excised tissues (Denison

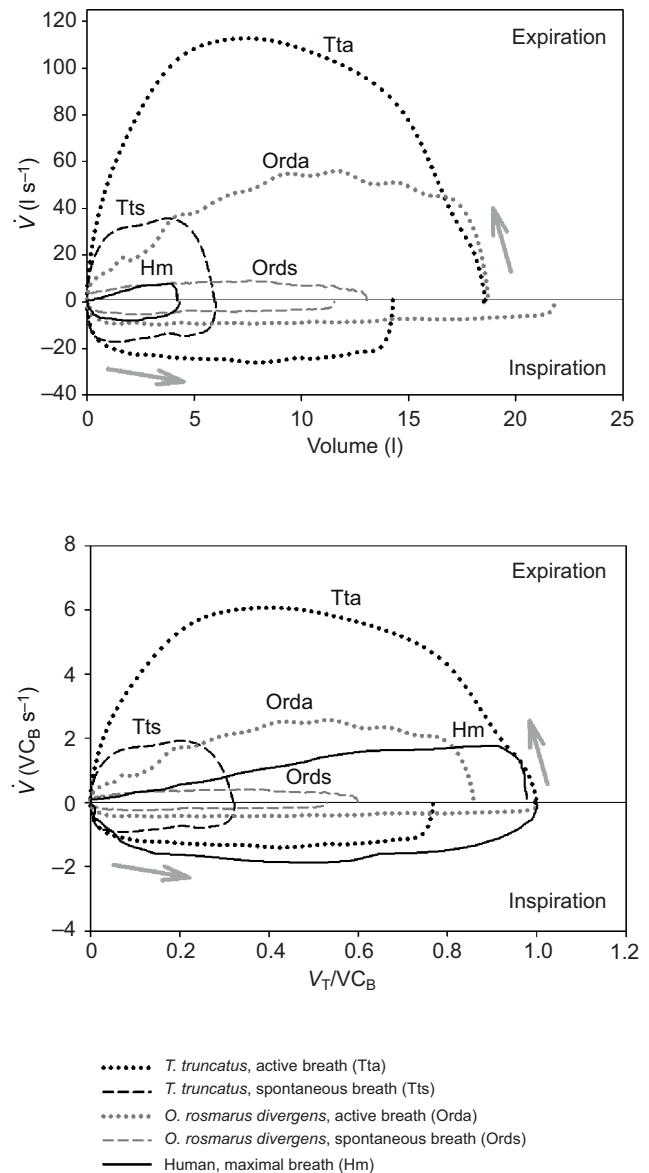


Fig. 3. Comparative flow-volume relationship for adult Pacific walrus, bottlenose dolphin and human. Data from one adult female Pacific walrus (*O. rosmarus divergens*) in the present study (animal ID 26005390, year of birth 2003, 821±38 kg), one adult male bottlenose dolphin (*Tursiops truncatus*, age 21 years, 197 kg; Fahlman et al., 2015), and one adult man (fig. 4 of Miller et al., 2005; reproduced with permission of the ERS © 2020). (A) Flow–volume relationship for spontaneous and active respiratory manoeuvres from both species of marine mammals, and for one maximal respiratory manoeuvre from the adult human male. (B) For the same data, respiratory flow and V_T were normalized by the maximum measured V_T for the represented active manoeuvre (walrus 21.8 l, dolphin 18.6 l, human 4.3 l), which was considered as the behavioural vital capacity (VC_B). For each representation, the arrow on the right shows the direction of the expiratory phase at the beginning of the respiratory manoeuvres (positive values of respiratory flow or normalized respiratory flow), while the arrow on the left shows the inspiratory phase (negative values). Spontaneous and active breaths were collected through voluntary participation of the animals, where the walrus was sitting and supported by the pectoral flippers and the dolphin was floating in water.

and Kooyman, 1973; Denk et al., 2020; Fahlman et al., 2011, 2014; Kooyman and Sinnett, 1979; Leith et al., 1972; Moore et al., 2011), which may not reflect respiratory function in a realistic biological scenario (Fahlman et al., 2017). Other studies have used restrained

Table 5. Summary of normalized respiratory variables for a number of resting marine mammals

Species	N	Stage	M _b (kg)	TLC _{est} (l)	sV _T (ml kg ⁻¹)	%TLC _{est}	sC _L (cmH ₂ O ⁻¹)	C _L /TLC _{est} (cmH ₂ O ⁻¹)	sV _E (l min ⁻¹ kg ⁻¹)	V _E /TLC _{est} (min ⁻¹)	sV _{exp} (s ⁻¹)	References
Human		Adult	70	4.8	9.1	14	0.07	0.04	0.17	2.5	2.3	Radeos and Camargo (2004); Stahl (1967)
Walrus	3	Adult	596–1064*	63.2±8.3	13.9±5.2	3–43 (50)	0.32±0.07 ¹	0.02 ¹	0.11±0.03	1.3±0.34	0.86	Present study
Grey seal ^{††}	4	Adult	140–250	17.3	32.3	35–49			0.63	7.1	0.57 [‡]	Reed et al. (1994)
Weddell seal	3	Subadult	260–345**	24.8	17.0	20 (46 ^{††})			0.11	1.3	0.29 ^{††}	Falke et al. (2008)
California sea lion	13	Adult and juvenile	49–130	7.4	25.1±1.7	26–30 (86 [§])			0.16	1.7	~3.9 [§]	Fahlman et al. (2020b); Kerem et al. (1975)
	4	Adult and juvenile (anaesthetized)	17–126	5.5±4.2			0.92±0.16 ⁴	0.07 ⁴				Fahlman et al. (2014)
Patagonia sea lion	5	Adult	94–286	13.4	28.5	47–73	0.41±0.11 ⁴	0.03 ⁴	0.13	1.4		Fahlman and Madigan (2016)
Bottlenose dolphin	6	Adult	158–250	16.8	30.8	34 (106)	0.31±0.04 ⁶	0.02 ⁶	0.10	1.2	8.0	Fahlman et al. (2015)
Beluga	9	Adult	510–891**	54.6	25.5	14–34 (97)			0.11	1.4	4.7	Fahlman et al. (2019b)

Common name (Species), number of studied individuals (N), reproductive stage (Stage), measured body mass (M_b) range and estimated total lung capacity (TLC_{est}=0.135M_b^{0.92}; Kooyman, 1973) from previous studies in marine mammals. Estimated values for an adult male human (M_b=70 kg, height 175 cm, age 40 years) are reported as a representation of terrestrial mammals. Listed values or ranges have been estimated from previous measured variables, while measured ranges or means (±s.d.) are provided when available. For tidal volume (V_T) and respiratory flow (V_E), we preferentially report data for the expiratory phase when available. For spontaneous breaths while resting, we report mass-specific tidal volume (sV_T), percentage of TLC_{est} reached (%TLC_{est}), specific lung compliance (sC_L), lung compliance divided by the minimum air volume, (MAV), lung compliance normalized by TLC_{est} (C_L/TLC_{est}), mass-specific respiratory minute volume (sV_E), and respiratory minute volume (V_E) normalized by TLC_{est} (V_E/TLC_{est}). For collected data from respiratory efforts, we additionally estimated the %TLC_{est} (values in parentheses), and the specific expiratory flow (sV_{exp}) normalized by the TLC_{est} for the reported maximal respiratory manoeuvre. *Range of measured M_b through the entire experimental period in the present study. Superscript numbers indicate the number of individuals that underwent lung compliance measurements. **All or some M_b values were estimated. [†]Estimated from the average for maximal reported manoeuvres. [‡]Data collected during surface periods after breath-holds. [§]Measured from a 2 year old, 32 kg subject from Kerem et al. (1975).

and/or involuntarily participating animals (Falke et al., 2008; Gallivan, 1981; Irving et al., 1941; Kerem et al., 1975; Kooyman et al., 1971; Olsen et al., 1969; Reed et al., 1994; Scholander and Irving, 1941; Spencer et al., 1967; Wahrenbrock et al., 1974), probably resulting in stress, which may affect the physiology and breathing patterns. As one important aspect when studying physiology is to minimize confounding variables during data collection, studies on trained animals may help minimize stress during voluntary participation (Fahlman et al., 2017). However, one disadvantage when working with trained animals is that voluntary compliance may differ between days. Also, the influence of the trainer may alter the breathing pattern (e.g. anticipatory behaviour could increase respiratory frequency), causing a bias that has to be assessed. For this reason, the subjects should undergo desensitization to the experimental procedures to ensure that data collection is minimally affected. In addition, it is also important to critically evaluate the data and assess whether the measurement has influenced the subject.

General physiological state of the study subjects

In the present study, the walrus participated in a total of 120 trials to ensure desensitization to the lung function procedure. A subset of these trials was analysed and reported where the animals were calm and breathing normally. Measured f_R during the experiments in the present study (7.6 ± 2.2 breaths min^{-1}) was higher than that estimated in resting semi-aquatic and fully aquatic mammals ($f_R = 33M_b^{-0.42}$; Mortola and Limoges, 2006), but lower than that predicted for terrestrial mammals of the same size ($f_R = 53.5M_b^{-0.26}$; Stahl, 1967). These measurements were close to data obtained from the same animals while floating inside a respirometer (5.9 ± 2.3 breaths min^{-1} ; A.B.-E. and A.F., unpublished observations), and to those reported in a single walrus under human care (5.9 breaths min^{-1} ; Mortola and Seguin, 2009).

The respiratory minute volume ($\dot{V}_E = f_R V_T$, l min^{-1}) is commonly used as a measure of the volume of air that is moved in and out of the lungs, and provides an alternative method to evaluate departure from normal ventilatory patterns and whether an increase in f_R results in hyperventilation (Prakash, 2015). For the three walrus participating in the present study, we found similar results when comparing the average mass-specific respiratory minute volume ($s\dot{V}_E$, $\text{l min}^{-1} \text{kg}^{-1}$) during exhalation with estimated $s\dot{V}_E$ calculated from previous reported data in resting trained marine mammals that were additionally monitored through focal observations (Table 5). Thus, the estimated $s\dot{V}_E$ suggests that the walrus were not hyperventilating but may have increased f_R during some experimental trials (see f_R ranges in Table 1), while at the same time reducing V_T to retain a normal \dot{V}_E and alveolar minute ventilation. This could also explain the wide range of measured V_T from spontaneous breaths (3–43% of TLC_{est}) when compared with previous studies in marine mammals (Table 5), and the obtained mass-exponent for f_R (Table 4) when compared with previous allometric equations (Fahlman et al., 2020a; Mortola and Limoges, 2006). We suggest that the variability in the reported f_R could be a sign of anticipatory behaviour caused by unintentional conditioning, as the animals were positively reinforced for complete breaths during the desensitization period. Despite this, the average and ranges for respiratory variables in the present study agree with those previously reported for marine mammals. Thus, although working with trained animals could result in a bias that should be considered and critically evaluated, the use of animals in managed care allows for data collection in a controlled environment where animal welfare is a priority. In addition, voluntary

participation of trained animals provides a manageable opportunity to obtain measurements that may be difficult and/or ethically challenging to collect from wild megafauna.

The effect of body position

Walrus, like other semi-aquatic species, spend part of their time on land and their respiratory function should be adapted to the two different media. When pinnipeds are lying on land, gravity could be affecting lung function as a result of increased pressure on the thoracic cage (Fahlman and Madigan, 2016). Similarly, the hydrostatic pressure of the water column on the chest could also affect lung function when floating at the water surface, as recently suggested for California sea lions (Fahlman et al., 2020b). We therefore tested lung function in water and on land (sitting and lying) to assess changes in lung function in the Pacific walrus. Obtained sV_T , T_{tot} and f_R remained the same for the three body positions, whereas measured \dot{V} was higher when the animals were floating in water as compared with lying down. Thus, obtained \dot{V}_{exp} and \dot{V}_{insp} are similar to those previously reported for California sea lions, where it was suggested that the increased hydrostatic pressure on the chest helped to increase \dot{V} , while T_{tot} decreased to achieve the same V_T and alveolar ventilation as on land (Fahlman et al., 2020b). While in the present study T_{tot} did not change with body position, the higher \dot{V} obtained when floating in water agrees with the previously suggested pressure effect of the water column.

In marine mammals, the expiratory phase is passive and mainly driven by the elastic recoil of the chest, while the inspiratory phase is active (Fahlman et al., 2017), and respiratory function could be limited by gravity and the increased pressure on the chest when lying on land. Indeed, the reported \dot{V} while lying on land in the present study was lower than when floating in water, which could suggest a possible flow limitation on land. However, the hydrostatic pressure could help assist elastic recoil during expiration, allowing for a passive increase in \dot{V}_{exp} while floating in water, as previously suggested (Fahlman et al., 2020b). While the results of the present study do not provide sufficient evidence of a gravitational alleviation while sitting as compared with lying, we do not discard this possible effect. Therefore, further studies on semi-aquatic species would help confirm the respiratory flow–volume limitations related to the body position and media location in these species.

Tidal volume and compliance

Previous studies have showed that marine mammals have more compliant lungs (i.e. higher C_L) and a more flexible chest compared with terrestrial mammals (Table 5) (Fahlman et al., 2017; Olsen et al., 1969; Piscitelli et al., 2013). These anatomical features allow these species to exchange much of their TLC in a single breath, and their VC is close to TLC (Fahlman et al., 2011, 2017; Kooyman and Sinnett, 1979; Piscitelli et al., 2010). However, some studies have shown that, even during respiratory efforts following dives or exercise, V_T for most breaths is below VC and only around 20–40% of TLC_{est} (see Table 5 in the present study and fig. 4 in Fahlman et al., 2020b). The measured V_T for spontaneous breaths in the present study was lower when compared with the estimated V_T for a number of marine mammals (Fig. 2), but was between 3% and 43% of TLC_{est} , and reached 50% of TLC_{est} when performing active respiratory manoeuvres. This range for spontaneous V_T is similar to that previously measured in resting marine mammals ranging from 20 to 3600 kg ($V_T=32\text{--}43\%$ of TLC_{est} ; Fahlman et al., 2017; Kooyman, 1973), and exceeded the 14% of TLC reported for terrestrial mammals (Table 5). In addition, the measured V_T for

spontaneous breaths was higher than that estimated for terrestrial mammals (Fig. 2), and correlated with M_b with a mass-exponent close to unity (Table 4) as previously reported in otariids (Fahlman and Madigan, 2016; Fahlman et al., 2020b) and cetaceans (Fahlman et al., 2020a). Similarly, the average measured sV_T for spontaneous breaths was higher as compared with that of terrestrial mammals, but was lower than that previously reported (Table 5) and estimated from a number of marine mammals (22 ml kg^{-1} ; Mortola and Seguin, 2009).

The measured dynamic sC_L from one animal in the present study was higher as compared with previous estimations for land mammals, as previously described in their marine counterparts (see Table 5 in the present study, and table 2 in Fahlman et al., 2017, for more species). While chest compliance was not measured in this study, previous results have shown that the chest in pinnipeds does not significantly contribute to the dynamic values (Fahlman et al., 2014). Thus, our results are consistent with previous studies that suggested an increased ventilatory capacity in marine mammals with larger V_T and dynamic sC_L as compared with terrestrial mammals, and that most breaths while resting or following active respiratory manoeuvres do not reach TLC.

Respiratory flow and flow–volume relationships

In addition to a flexible thorax and compliant lungs, previous studies on the anatomy and mechanical properties of the respiratory system in marine mammals showed that many species have reinforced conducting airways (Bagnoli et al., 2011; Cozzi et al., 2005; Denison and Kooyman, 1973; Fahlman et al., 2017; Kooyman, 1973; Moore et al., 2014; Piscitelli et al., 2013). These anatomical features would allow for alveolar compression during diving and also adequate gas exchange during high \dot{V}_{exp} and short T_{tot} as compared with terrestrial mammals (Fahlman et al., 2017; Kooyman and Sinnett, 1982; Piscitelli et al., 2010; Stahl, 1967). However, when comparing \dot{V}_{exp} and T_{tot} in marine mammals, there appears to be considerable variability (Fahlman et al., 2017; Ponganis, 2011), possibly as a result of the large diversity in the respiratory anatomical and mechanical adaptations within this group of mammals (Fahlman et al., 2017; Kooyman, 1973; Moore et al., 2014; Piscitelli et al., 2010, 2013). The peak $s\dot{V}$ can be used to compare the ventilatory exchange capacity among different species, and previous studies have showed that some cetaceans and the California sea lion exceed reported $s\dot{V}$ for humans (Table 5). While measured \dot{V}_{exp} during active manoeuvres in the present study was higher than that reported for humans (Fig. 3), the maximal peak $s\dot{V}_{\text{exp}}$ was between the reported values for humans, cetaceans and the California sea lion and those estimated from available data of phocids (Table 5). Further studies providing the opportunity to correlate mechanical properties and respiratory function in these species would help us to understand their respiratory adaptations and the functional consequences on their exchange capacity.

The reported flow–volume relationships for the Pacific walrus in the present study indicated that the flow during active and spontaneous exhalation is maintained over most of the V_T , as previously reported for marine mammals (Fig. 3) (Borque-Espinosa et al., 2020; Fahlman et al., 2019b; Fahlman et al., 2015; Fahlman and Madigan, 2016; Kerem et al., 1975; Kooyman and Cornell, 1981; Kooyman et al., 1975; Kooyman and Sinnett, 1979; Matthews, 1977; Olsen et al., 1969). In contrast, the respiratory mechanics of humans show that the peak expiratory flow during maximal exhalations is effort independent, where the peak occurs at high lung volumes and rapidly drops while lung volume decreases (Fig. 3) (Hyatt et al., 1958; Jordanoglou and Pride, 1968). This flow

limitation in humans is related to the increasing flow resistance caused by the compression of the flexible distal airways during emptying of the lungs (Hyatt et al., 1958). Thus, considering the stiffer airways described for marine mammals and the reported flow–volume relationships, it is likely that the flow is not limited by the conducting airways and that the expiration during active and spontaneous respiratory manoeuvres appears to be effort dependent in this group of mammals. However, in a recent study that aimed to assess respiratory health in bottlenose dolphins (*Tursiops truncatus*) similar to spirometry methods in humans (Clausen, 1982; Crapo, 1994), the flow–volume relationships showed flow limitations associated with obstructive respiratory disease (Borquez-Espinosa et al., 2020). While additional studies should be conducted to determine whether this also translates to other marine mammals, we expect that respiratory disease would have similar consequences in other species considering the respiratory similarities among this group. Therefore, increased baseline information of normal lung function and flow–volume dynamics in these species would allow evaluation of the mechanical consequences of respiratory disease. This would be beneficial in terms of gaining a better understanding about the respiratory function limitations in marine mammals, and would enhance our effort in the protection of these species through the development of new diagnostic methods.

Conclusions

With the results presented in the current study, we have provided additional data about the respiratory capacity in another marine mammal, the walrus, which will add to collective information to improve our understanding of respiratory physiology in marine mammals. While we collected data on three adult Pacific female walruses, additional information from animals of different age and sex would be relevant to help improve the understanding of respiratory physiology in this species. The results presented here are in agreement with those reported in California sea lions and suggest a flow limitation when lying on land, and that hydrostatic pressure could help increase \dot{V}_{exp} when resting in water versus on land. Further studies would help confirm the respiratory function limitations when lying on land as compared with sitting. Consequently, we propose that lung function studies in other semi-aquatic marine mammals should evaluate respiratory function both on land and in water.

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Competing interests

The authors declare no competing or financial interests.

Author contributions

Conceptualization: A.B.-E., A.F.; Methodology: A.B.-E., D.F.-F., A.F.; Software: A.F.; Validation: A.B.-E., A.F.; Formal analysis: A.B.-E., A.F.; Investigation: A.B.-E., D.F.-F., A.F.; Resources: A.F.; Data curation: A.B.-E., A.F.; Writing - original draft: A.B.-E.; Writing - review & editing: D.F.-F., R.C.-A., A.F.; Visualization: A.B.-E.; Supervision: R.C.-A., A.F.; Project administration: A.F.; Funding acquisition: A.F.

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Data availability

The data used in this study are freely available from the Open Science Framework: <https://osf.io/qd4cy>.

References

- Bagnoli, P., Cozzi, B., Zaffora, A., Acocella, F., Fumero, R. and Costantino, M. L. (2011). Experimental and computational biomechanical characterization of the tracheo-bronchial tree of the bottlenose dolphin (*Tursiops truncatus*). *J. Biomech.* **44**, 1040–1045. doi:10.1016/j.jbiomech.2011.02.005
- Bertelsen, M. F., Acquarone, M. and Born, E. W. (2006). Resting heart and respiratory rate in wild adult male walruses (*Odobenus rosmarus rosmarus*). *Mar. Mamm. Sci.* **22**, 714–718. doi:10.1111/j.1748-7692.2006.00055.x
- Borquez-Espinosa, A., Burgos, F., Dennison, S., Laughlin, R., Manley, M., Capaccioni Azzati, R. and Fahlman, A. (2020). Pulmonary function testing as a diagnostic tool to assess respiratory health in bottlenose dolphins *Tursiops truncatus*. *Dis. Aquat. Org.* **138**, 17–27. doi:10.3354/dao03447
- Bostrom, B. L., Fahlman, A. and Jones, D. R. (2008). Tracheal compression delays alveolar collapse during deep diving in marine mammals. *Respir. Physiol. Neurobiol.* **161**, 298–305. doi:10.1016/j.resp.2008.03.003
- Burki, N. K. (1981). Spirometry and other pulmonary function tests. *J. Fam. Pract.* **12**, 119–124.
- Clausen, J. L. (1982). *Pulmonary Function Testing Guidelines and Controversies*. New York, NY: Academic Press.
- Cozzi, B., Bagnoli, P., Acocella, F. and Costantino, M. L. (2005). Structure and biomechanical properties of the trachea of the striped dolphin *Stenella coeruleoalba*: evidence for evolutionary adaptations to diving. *Anat. Rec. Part A Discover. Mol. Cell Evol. Biol.* **284A**, 500–510. doi:10.1002/ar.a.20182
- Crapo, R. O. (1994). Pulmonary-function testing. *N. Engl. J. Med.* **331**, 25–30. doi:10.1056/NEJM199407073310107
- Denison, D. M. and Kooyman, G. L. (1973). The structure and function of the small airways in pinniped and sea otter lungs. *Respir. Physiol.* **17**, 1–10. doi:10.1016/0034-5687(73)90105-9
- Denk, M., Fahlman, A., Dennison-Gibby, S., Song, Z. and Moore, M. (2020). Hyperbaric tracheobronchial compression in cetaceans and pinnipeds. *J. Exp. Biol.* **223**, jeb217885. doi:10.1242/jeb.217885
- Fahlman, A. and Madigan, J. (2016). Respiratory function in voluntary participating Patagonia sea lions (*Otaria flavescens*) in sternal recumbency. *Front. Physiol.* **7**, 1–9. doi:10.3389/fphys.2016.00528
- Fahlman, A., Hooker, S. K., Olszowka, A., Bostrom, B. L. and Jones, D. R. (2009). Estimating the effect of lung collapse and pulmonary shunt on gas exchange during breath-hold diving: the Scholander and Kooyman legacy. *Respir. Physiol. Neurobiol.* **165**, 28–39. doi:10.1016/j.resp.2008.09.013
- Fahlman, A., Loring, S. H., Ferrigno, M., Moore, C., Early, G., Niemeyer, M., Lentell, B., Wenzel, F., Joy, R. and Moore, M. J. (2011). Static inflation and deflation pressure-volume curves from excised lungs of marine mammals. *J. Exp. Biol.* **214**, 3822–3828. doi:10.1242/jeb.056366
- Fahlman, A., Loring, S. H., Johnson, S. P., Haulena, M., Trites, A. W., Fravel, V. A. and Van Bonn, W. G. (2014). Inflation and deflation pressure-volume loops in anesthetized pinnipeds confirms compliant chest and lungs. *Front. Physiol.* **5**, 1–7. doi:10.3389/fphys.2014.00433
- Fahlman, A., Loring, S. H., Levine, G., Rocho-Levine, J., Austin, T. and Brodsky, M. (2015). Lung mechanics and pulmonary function testing in cetaceans. *J. Exp. Biol.* **218**, 2030–2038. doi:10.1242/jeb.119149
- Fahlman, A., Moore, M. J. and Garcia-Parraga, D. (2017). Respiratory function and mechanics in pinnipeds and cetaceans. *J. Exp. Biol.* **220**, 1761–1773. doi:10.1242/jeb.126870
- Fahlman, A., Brodsky, M., Miedler, S., Dennison, S., Ivančić, M., Levine, G., Rocho-Levine, J., Manley, M., Rocabert, J. and Borquez-Espinosa, A. (2019a). Ventilation and gas exchange before and after voluntary static surface breath-holds in clinically healthy bottlenose dolphins, *Tursiops truncatus*. *J. Exp. Biol.* **222**, jeb.192211. doi:10.1242/jeb.192211
- Fahlman, A., Epple, A., García-Parraga, D., Robeck, T., Haulena, M., Piscitelli-Doshkov, M. and Brodsky, M. (2019b). Characterizing respiratory capacity in belugas (*Delphinapterus leucas*). *Respir. Physiol. Neurobiol.* **260**, 63–69. doi:10.1016/j.resp.2018.10.009
- Fahlman, A., Borquez-Espinosa, A., Facchin, F., Fernandez, D. F., Caballero, P. M., Haulena, M. and Rocho-Levine, J. (2020a). Comparative respiratory physiology in cetaceans. *Front. Physiol.* **11**, 1–7. doi:10.3389/fphys.2020.00142
- Fahlman, A., Meegan, J., Borquez-Espinosa, A. and Jensen, E. D. (2020b). Pulmonary function and resting metabolic rates in California sea lions (*Zalophus californianus*) on land and in water. *Aquat. Mamm.* **46**, 67–79. doi:10.1578/AM.46.1.2020.67
- Falke, K. J., Busch, T., Hoffmann, O., Liggins, G. C., Liggins, J., Mohnhaupt, R., Roberts, J. D., Jr., Stanek, K. and Zapol, W. M. (2008). Breathing pattern, CO₂ elimination and the absence of exhaled NO in freely diving Weddell seals. *Respir. Physiol. Neurobiol.* **162**, 85–92. doi:10.1016/j.resp.2008.04.007

- Frederick, B. N. (1999). Fixed-, Random-, and Mixed-Effects ANOVA Models: A User-Friendly Guide for Increasing the Generalizability of ANOVA Results. Institute of Education Sciences, ERIC Number: ED426098.
- Gallivan, G. J. (1981). Ventilation and gas exchange in unrestrained harp seals (*Phoca groenlandica*). *Comp. Biochem. Phys. A* **69**, 809-813. doi:10.1016/0300-9629(81)90175-4
- Hyatt, R. E., Schilder, D. P. and Fry, D. L. (1958). Relationship between maximum expiratory flow and degree of lung inflation. *J. Appl. Physiol.* **13**, 331-336. doi:10.1152/jappl.1958.13.3.331
- Irving, L. (1939). Respiration in diving mammals. *Physiol. Rev.* **19**, 112-134. doi:10.1152/physrev.1939.19.1.112
- Irving, L., Scholander, P. F. and Grinnell, S. W. (1941). The respiration of the porpoise, *Tursiops truncatus*. *J. Cell. Physiol.* **17**, 145-168. doi:10.1002/jcp.1030170203
- Jordanoglou, J. and Pride, N. B. (1968). Factors determining maximum inspiratory flow and maximum expiratory flow of the lung. *Thorax*. **23**, 33-37. doi:10.1136/thx.23.1.33
- Kerem, D. H., Kylstra, J. A. and Saltzman, H. A. (1975). Respiratory flow rates in the sea lion. *Undersea Biomed. Res.* **2**, 20-27.
- Kooyman, G. L. (1973). Respiratory adaptations in marine mammals. *Am. Zool.* **13**, 457-468. doi:10.1093/icb/13.2.457
- Kooyman, G. L. and Cornell, L. H. (1981). Flow properties of expiration and inspiration in a trained bottle-nosed porpoise. *Physiol. Zool.* **54**, 55-61. doi:10.1086/physzool.54.1.30155804
- Kooyman, G. L. and Sinnett, E. E. (1979). Mechanical properties of the harbor porpoise lung, *Phocoena phocoena*. *Respir. Physiol.* **36**, 287-300. doi:10.1016/0034-5687(79)90042-2
- Kooyman, G. L. and Sinnett, E. E. (1982). Pulmonary shunts in Harbor seals and sea lions during simulated dives to depth. *Physiol. Zool.* **55**, 105-111. doi:10.1086/physzool.55.1.30158447
- Kooyman, G. L., Kerem, D. H., Campbell, W. B. and Wright, J. J. (1971). Pulmonary function in freely diving Weddell seals, *Leptonychotes weddelli*. *Respir. Physiol.* **12**, 271-282. doi:10.1016/0034-5687(71)90069-7
- Kooyman, G. L., Norris, K. S. and Gentry, R. L. (1975). Spout of the gray whale: its physical characteristics. *Science*. **190**, 908-910. doi:10.1126/science.190.4217.908
- Leith, D. E., Lowe, R. and Gillespie, J. (1972). Mechanics of baleen whale lungs. *Fed. Proc.* **31**, 335.
- Littell, R. C., Henry, P. R. and Ammerman, C. B. (1998). Statistical analysis of repeated measures data using SAS procedures. *J. Anim. Sci.* **76**, 1216-1231. doi:10.2527/1998.7641216x
- Matthews, R. C. (1977). *Pulmonary Mechanics of California sea Lions, Zalophus californianus*, Vol. MSc. San Diego: San Diego State University.
- Miller, M. R., Hankinson, J., Brusasco, V., Burgos, F., Casaburi, R., Coates, A., Crapo, R., Enright, P., van der Grinten, C. P. M., Gustafsson, P. et al. (2005). Standardisation of spirometry. *Eur. Respir. J.* **26**, 319-338. doi:10.1183/09031936.05.00034805
- Moore, M. J., Hammar, T., Arruda, J., Cramer, S., Dennison, S., Montie, E. and Fahlman, A. (2011). Hyperbaric computed tomographic measurement of lung compression in seals and dolphins. *J. Exp. Biol.* **214**, 2390-2397. doi:10.1242/jeb.055020
- Moore, C., Moore, M. J., Trumble, S., Niemeyer, M., Lentell, B., McLellan, W., Costidis, A. and Fahlman, A. (2014). A comparative analysis of marine mammal tracheas. *J. Exp. Biol.* **217**, 1154-1166. doi:10.1242/jeb.093146
- Mortola, J. P. and Limoges, M.-J. (2006). Resting breathing frequency in aquatic mammals: a comparative analysis with terrestrial species. *Respir. Physiol. Neurobiol.* **154**, 500-514. doi:10.1016/j.resp.2005.12.005
- Mortola, J. P. and Seguin, J. (2009). End-tidal CO₂ in some aquatic mammals of large size. *Zoology* **112**, 77-85. doi:10.1016/j.zool.2008.06.001
- Olsen, C. R., Hale, F. C. and Elsner, R. (1969). Mechanics of ventilation in the pilot whale. *Respir. Physiol.* **7**, 137-149. doi:10.1016/0034-5687(69)90001-2
- Piscitelli, M. A., McLellan, W. A., Rommel, S. A., Blum, J. E., Barco, S. G. and Pabst, D. A. (2010). Lung size and thoracic morphology in shallow- and deep-diving cetaceans. *J. Morphol.* **271**, 654-673. doi:10.1002/jmor.10823
- Piscitelli, M. A., Raverty, S. A., Lillie, M. A. and Shadwick, R. E. (2013). A review of cetacean lung morphology and mechanics. *J. Morphol.* **274**, 1425-1440. doi:10.1002/jmor.20192
- Ponganis, P. J. (2011). Diving mammals. *Compr. Physiol.* **1**, 447-465. doi:10.1002/cphy.c091003
- Prakash, E. S. (2015). What is the best definition of the term "hyperventilation"? *Adv. Physiol. Educ.* **39**, 137-138. doi:10.1152/advan.00078.2014
- Quanjer, P. H., Tammeling, G. J., Cotes, J. E., Pedersen, O. F., Peslin, R. and Yernault, J.-C. (1993). Lung volumes and forced ventilatory flows. *Eur. Respir. J.* **6**, 5-40. doi:10.1183/09041950.005s1693
- Radeos, M. S. and Camargo, C. A., Jr. (2004). Predicted peak expiratory flow: differences across formulae in the literature. *Am. J. Emerg. Med.* **22**, 516-521. doi:10.1016/j.ajem.2004.08.018
- Reed, J. Z., Chambers, C., Fedak, M. A. and Butler, P. J. (1994). Gas exchange of captive freely diving grey seals (*Halichoerus grypus*). *J. Exp. Biol.* **191**, 1-18.
- Reed, J. Z., Chambers, C., Hunter, C. J., Lockyer, C., Kastelein, R., Fedak, M. A. and Boutilier, R. G. (2000). Gas exchange and heart rate in the harbour porpoise, *Phocoena phocoena*. *J. Comp. Physiol. B* **170**, 1-10. doi:10.1007/s003600050001
- Scholander, P. F. (1940). Experimental investigations on the respiratory function in diving mammals and birds. *Hvalrådets Skrifter* **22**, 1-131.
- Scholander, P. F. and Irving, L. (1941). Experimental investigations on the respiration and diving of the Florida manatee. *J. Cell. Physiol.* **17**, 169-191. doi:10.1002/jcp.1030170204
- Spencer, M. P., Thomas, A., Gornall, T. A. and Poulter, T. C. (1967). Respiratory and cardiac activity of killer whales. *J. Appl. Physiol.* **22**, 974-981. doi:10.1152/jappl.1967.22.5.974
- Stahl, W. R. (1967). Scaling of respiratory variables in mammals. *J. Appl. Physiol.* **22**, 453-460. doi:10.1152/jappl.1967.22.3.453
- Stirling, I. and Sjare, B. (1988). Preliminary observations on the immobilization of male atlantic walruses (*Odobenus rosmarus rosmarus*) with Telazol®. *Mar. Mamm. Sci.* **4**, 163-168. doi:10.1111/j.1748-7692.1988.tb00196.x
- Wahrenbrock, E. A., Maruscha, G. F., Elsner, R. and Kenney, D. W. (1974). Respiration and metabolism in 2 baleen whale calves. *Mar. Fish. Rev.* **36**, 3-9.